2. On page 2, last paragraph, (l. 34 spilling over to p. 3, l. 12), please delete this paragraph and insert the following in its place:

Studies from a number of laboratories have characterized the ability of the endothelial cell to dramatically alter basic activities in response to cytokines such as tumor [3]necrosis factor (TNF) -alpha. TNF-alpha stimulation induces significant alterations in the production of vasoactive compounds such as nitric oxide and endothelin, increases surface stickiness toward various types of leukocytes, and modulates the expression of both pro- and anti-coagulant factors (Cotran et al., 1990, *J. Am. Soc. Nephrol.* 1:225-235; Mantovani et al., 1992, *FASEB J.* 6:2591-2599). In turn, endothelial cells have been shown to be an important source for the production of cytokines and hormones, including interleukin 1, 6 and 8 (Gimbrone et al., 1989, *Science* 246:1601-1603; Locksley et al. 1987, *J. Immunol.* 139:1891-1895; Loppnow *et al.*, 1989, *Lymphokine. Res.* 8:293-299; Warner et al., 1987, *J. Immunol.* 139:1911-1917).

3. On page 9, ll. 8-31, please delete the brief descriptions for "Figure 2," "Figure 3A-3E," and "Figure 4A-4C" and insert in their places the following:

Figure 2. Homology analysis between the deduced amino acid sequence of the putative *del-1* gene (m-del1) (SEQ ID NO: 1) and other proteins with "discoidin-like domains." Identical residues are boxed, conserved -residues are shaded (Geneworks, Intelligenetics, Mountain View, CA). m-*del-1* sequence (SEQ ID NO: 1) was derived from a trapped exon and mouse embryo

cDNAs. Abbreviations:h-MFG, human milk fat globule protein (SEQ ID NO: 2); h-FV, human coagulation factor V (SEQ ID NO: 3); m-FVIII, mouse coagulation factor VIII (SEQ ID NO: 4); X-A5b1 (SEQ ID NO: 5) and X-A5b2 (SEQ ID NO: 6), b1 and b2 domains of Xenopus neuronal antigen A5; dis-I, discoidin I (SEQ ID NO: 7); consensus sequence (SEQ ID NO: 8).

Figure 3A-[3E] 3D. Nucleotide sequence and deduced amino acid sequence of murine *del-1* cDNA (SEQ ID NO: 9) and (SEQ ID NO: 10).

Figure 4A-4C. Nucleotide sequence and deduced amino acid sequence of sequence of human *del-1* cDNA (SEQ ID NO: 11) and (SEQ ID NO: 14).

4. On page 10, ll. 1-6, please delete the brief description for "Figure 6" and insert in its place the following:

Figure 6. Amino acid sequence comparison between murine (m-*del-1*) (SEQ ID NO: 10) and human (h-*del-1*) [(SEQ ID NO: 29)] (SEQ ID NO: 30) Del-1-proteins. The EGF-like and discoidin-like domains are indicated by "egf" and "discoidin," respectively.

5. On page 10, ll. 33-37, please delete the brief description for "Figure 8" and insert in its place the following:

Figure 8. The 54.2% amino acid homology between human Del-1 (SEQ ID

NO:21) and MFG-E8 (SEQ ID NO: [21] 20) in the tandem discoidin I/factor VIII domains is shown. These domains are rich in the basic amino acids arginine and lysine. The 5' domain contains 12 arginines and 12 lysines versus 9 acidic residues, while the 3' domain contains 8 arginines and 10 lysines versus 16 acidic residues. A similar domain in the coagulation factor VIII protein is believed to bind to negatively charged phospholipids on the surface of platelets. The MFG-E8 protein has been found to associate tightly with milk fat globule membranes.

6. On page 12, ll. 4-6, please delete the brief description of "Figure 11" and insert in its place the following:

Figure 11. Human del-1 splicing variant partial sequence [(SEQ ID NO:27)] (SEQ ID NO: 31) showing the variation as compared with the major form (SEQ ID NO:30).

7. On page 12, ll. 8-10, please delete the brief description of "Figure 12A-12E" and insert in its place the following:

Figure 12A-12E. Murine *del-1* truncated minor nucleotide and deduced amino acid sequences (SEQ ID NO: 28) and (SEQ ID NO: 29).

8. On page 44, ll. 6-15, please delete the paragraph and insert in its place the following:

The anti-angiogenic activity of Del-1 may be used to treat abnormal conditions that result from angiogenesis. These conditions include, but are not limited to, cancer, diabetic retinopathy, rheumatoid arthritis and endometriosis.

Additionally, the removal or inhibition of Del-1 in situations where it naturally inhibits blood vessel formation may be used to promote angiogenesis. These conditions include, but are not limited to, cardiac ischemia, thrombotic stroke, [would] wound healing and peripheral vascular disease. Furthermore, Del-1 may be used to stimulate bone formation.

9. On page 47, please delete the paragraph starting on l. 4 spilling over to page 48 l. 2, and insert in its place the following:

A 160 bp exon was trapped from a fragment of genomic DNA located approximately 10 kb from the "left" integration site. Nucleotide sequence of the trapped exon was employed to screen various nucleic acid databanks through the BLAST routine at the NCBI, revealing no other gene with significant nucleic acid homology. The deduced amino acid sequence of the single open reading frame was subsequently employed in databank searches. These revealed that the protein domain encoded in the trapped exon was similar in part to domains in a number of proteins, including Factor V, Factor VIII and discoidin I (Figure 2, SEQ ID NOS 1-8) (Jenny et al., 1987, *Proc. Natl. Acad. Sci.* U.S.A. 84:4846-4850; Poole et al., 1981, *J. Mol. Biol.* 153:273-289; Toole et al., 1984, *Nature* 312:342-347) The protein which was most similar was milkfat globule protein, which had been found on the surface of mammary epithelial cells (1994, WO 94/11508). It has been hypothesized that the discoidin I-like domain in this protein allows it to localize to the surface of the epithelial cell (Larocca *et al.*, 1991, *Cancer Res.* 51:4994-4998; Stubbs *et al.*, 1990, *Proc. Natl. Acad. Sci. U.S.A.* 87:8417-8421). The homologous regions of Factor V and Factor VIII have been implicated in their interaction with phospholipids on the

surface of endothelial cells and platelets (Jenny *et al.*, 1987, *Proc. Natl. Acad. Sci. U.S.A.* 84:4846-4850; Toole *et al.*, 1984, *Nature* 312:342-347). Homology to the Xenopus protein A5 was also observed. A5 is a neuronal cell surface molecule which is expressed in retinal neurons and the neurons in the visual center with which the retinal neurons contact (Takagi *et al.*, 1991, *Neuron* 7:295-307). A5 has been proposed to play a role as a neuronal recognition molecule in the development of this neural circuit, perhaps through mediating intercellular signaling. The protein for which this discoidin I-like domain was named is a protein expressed in *Dictyostelium discoideum*, which serves an essential role in the aggregation of individual cells.

10. On page 48, ll. 3-27, please delete the two paragraphs and insert the following in their place:

The DNA fragment encoding the trapped exon was employed as a probe in a Southern blot experiment and shown to hybridize with regions of the *del-1* locus outside of the region that was employed in the exon trap construct. Given this finding, cDNA cloning was pursued by using the exon trap probe to screen an 11.5 day embryonic mouse cDNA library. Clones were plaque purified, and inserts subcloned into plasmid for further analysis. Nucleotide sequence analysis showed that two of the embryonic cDNA clones contained the sequence of the trapped exon. Sequence from the clones was used to expand the deduced amino acid sequence of the discoidin I-like domain (Figure 2, SEQ ID NOS 1-8). The full nucleotide sequence of these cDNAs was analyzed and cloned into plasmid vectors which allowed the generation of cRNA transcripts for RNAse protection and *in situ* hybridization (Figure 3A-3D[3E], SEQ ID NO: 9).

A human cDNA was isolated from a human fetal lung cDNA lambda phage library purchased from Clontech Inc. (Figure 4A-4C, SEQ ID NO: 11). A portion of the mouse *del-1* cDNA was used as a probe (Figure 5, SEQ ID NO: 19). The identity of the human cDNA clone was confirmed by comparing the human and mouse DNA sequences. These clones show approximately 80% DNA sequence homology and approximately 94% amino acid sequence homology (Figure 6, SEQ ID NOS: 10 and 30). These sequences are referred to as the "major" form of *del-1*. Upon initial isolation of *del-1*, standard molecular biology methods were used for isolating additional clones.

11. On page 48, please delete the paragraph starting from line 28 spilling over to page 49, line 3, and insert in its place the following:

DNA sequence analysis of the human *del-1* revealed an open reading frame of 1,446 base pairs predicted to encode a 481 amino acid protein with a molecular weight of, 53,797. The mouse cDNA encodes a 480 amino acid protein. Homology comparisons with DNA and protein databases indicated that the Del-1 protein was composed of three EGF-like protein domains, followed by two discoidin I/factor VIII-like domains (Figure 7). Genes similar to *del-1* included some key regulators of cell determination and differentiation such as Notch. Overall, the Del-1 protein has a structure similar to the membrane-associated milk fat globule membrane protein, MGF-E8, which has been used to develop antibodies for imaging breast cancer (Figure 8, SEQ ID NOS: 20 and 21).

12. On page 49, ll. 15-35, please delete the two paragraphs and insert in their places the following:

Key structural features of the open reading frame of human Del-1 include:

1) the presumed initiator methionine and putative secretion signal sequence (Figure 9, SEQ ID NO: 22)
2) the three EGF-like domains (Figure 10, SEQ ID NOS: 23-25)
3) the two discoidin I-like domains.

Further cloning and analysis of both the human and murine *del-1* genes revealed additional variant forms. For example, a human splicing variant (Z20 clone) was obtained in which 30 bp (*i.e.* 10 amino acids) (SEQ ID: 30 # 66-#75) between the first and second EGF-like domains of the major form (SEQ NO ID: 30) of *del-1* had been removed (Figure 11, SEQ ID NO: 31). In addition, a truncated version of murine *del-1* was isolated, which contained a signal peptide sequence, all three EGF-like domains and only a partial amino-terminal discoidin I/factor VIII-like domain (about 40%). This variant is referred to as murine *del-1* minor sequence, which is disclosed in Figure 12A-12D, SEQ ID NOS: 28 and 29.[12E] This transcript was cloned only from mouse embryonic libraries, but was verified through cloning of several independent cDNAs.

13. On page 50, please delete the paragraph starting at line 23 spilling over to page 51, line 6 and insert in its place the following:

In order to study the expression of: the *del-1* gene, Northern blots containing RNA obtained from a variety of human and mouse tissues (Clontech, Palo Alto, CA) were hybridized with a radiolabeled DNA probe as shown in Figure 5, SEQ ID NO: 19. In addition, adult organs, 15.5 dpc whole embryos and organs dissected from embryos were disrupted with a polytron, and RNA isolated over [C_sCl] CsCl gradient (Sambrook *et al.*, 1989, Molecular Cloning, Laboratory Manual, Cold Spring Harbor Laboratory, NY). Briefly, the blots were prehybridized at 42°C for 3-6 hours in a solution containing 5X SSPE, 10X Denhardt's solution, 100 μg/ml freshly denatured, sheared salmon sperm DNA, 50% formamide (freshly deionized), and 2% SDS. The radiolabeled probe was heat denatured and added to the prehybridization mix and allowed to hybridize at 42°C for 18-24 hours with constant shaking. The blots were